CONTROLLED MUTATION IN MAIZE

BARBARA MCCLINTOCK

Experimentation conducted during the past year was aimed at expanding our knowledge of the kinds of elements carried in the maize chromosomes that control gene action and give rise to changes in this action, that is, to mutations. Different controlling elements have been recognized, each characterized by its own specific mode of control of gene action and mutation; and this mode of control is quite independent of the primary type of action of the genic materials themselves, which these elements may serve to modify. Their presence in the chromosome complement is made evident because they undergo transposition from one location to another and do not lose their specificity of action in the process. Were it not for such behavior, these elements would remain undetected. Insertion of a controlling element at a locus of known gene action results in immediate or subsequent change in this action, or both. Each element expresses its own mode of control of change in gene action, and this allows the presence of the element at the locus to be recognized. The same element may be inserted at a number of different locations and thus come to control the action of genic materials at each of these locations. Conversely, the action of the same genic material may be influenced by different controlling elements, as a result of independent insertions of such elements at one particular locus.

Evidence of the control of gene action and mutation at a number of different known loci in maize by the *Ds-Ac* two-element controlling system has been reported in past years. Knowledge gained

from concentrated attention to the mode of operation of this particular system has provided the basic information that now serves as a guide in planning experimentation and in interpreting the modes of operation of other controlling elements or of systems of interrelated elements. With this framework of knowledge, advances in these studies may be made much more rapidly and effectively. In the past year, attention has been given to a system that differs considerably from the *Ds-Ac* system in its manner of control of gene action and mutation. A tentative hypothesis to account for the operation of this system was outlined in Year Book No. 53. Extensive tests have now been conducted, and the evidence obtained from them fully supports the hypothesis previously formulated. Much additional knowledge of the mode of operation of the system has also been obtained. A summary of this may now be given.

The a_1^{m-1}-*Spm* System of Control of Gene Action and Mutation

The A_1 locus in chromosome 3 of maize is a particularly favorable one for examining the operation of controlling systems. The genic materials at this locus are concerned with the development of anthocyanin pigmentation both in the plant tissues and in the aleurone layer of the kernel. When a change in intensity, quality, or pattern of distribution of that pigment appears in an individual plant or kernel, the altered phenotype is readily noticed. Therefore it is possible to detect insertions of controlling elements at the locus shortly after they occur. Different systems controlling gene action and mutation at this one locus have been recognized, and their modes of operation examined. Each was detected, initially, because of a distinct deviation from the standard A_1 type of expression of anthocyanin pigmentation, appearing either in an individual kernel on an ear or in an individual plant of a culture. Four of these systems have been analyzed in some detail and their modes of operation defined: the a_1-*Dt* (Dotted) system, the *Ds-Ac* system, the a_1^{m-1}-*Spm* system, and still another system operating at this locus to control the type and distribution of anthocyanin pigmentation in both kernel and plant. The system with which we shall be concerned in this section is the a_1^{m-1}-*Spm* system.

Knowledge of the behavior of controlling systems makes it evident that this one is composed of two interrelated but independently located elements. One of them, becoming inserted at the locus of A_1, caused a modification of the action of the genic materials located there. The modified locus has been designated a_1^{m-1} to distinguish it from other modifications that have arisen independently at this same locus. Subsequent changes have occurred at the locus, each effecting a change in gene action. These are regarded as arising from alterations of the controlling element. With a few possible exceptions, the genic materials themselves do not appear to be altered by such modifications. Their mode of action, however, may be decidedly altered as a result of any one change in the associated element. Thus the observed changes in gene action are referable, on the whole, to changes in the controlling element and not to irreversible changes in the gene substances. Moreover, such changes in gene action, stemming from alterations in the controlling element at the A_1 locus, can occur only when a second, independently located element is also present in the nucleus. The second element of this system is called Suppressor-mutator (symbolized as *Spm*), for the following reasons. In plants that either are homozygous for a_1^{m-1} or are a_1^{m-1}/a_1 in constitution, and that also have *Spm* in their nuclei, no anthocyanin pigment develops in the aleurone layer of the kernel or in the plant tissues until, in a somatic or a germinal cell, a modification of the controlling element located at A_1 allows pigment to be formed in those cells where it normally develops when the standard organization at the A_1 locus is present. These modifications effect

stable mutations, in that the altered type of gene action so produced continues to be expressed in subsequent cell and plant generations, both in the presence and in the absence of *Spm*. In plants of the above-named constitutions that do not have *Spm* in their nuclei, on the other hand, restricted gene action occurs, and this results in the appearance of uniformly distributed pigment both in the aleurone layer of the kernel and in the plant tissues. This expression of the genic substance at A_1 is constant and stable through successive plant generations as long as the *Spm* element is absent from the nuclei, for no mutations occur. Return of *Spm* to the nuclei, however, by appropriate crosses, again initiates the Suppressor-mutator effect on the element located at A_1. Gene action is again suppressed until, in a somatic or germinal cell, some change in this element allows the genic materials to function in some particular manner.

Unlike its counterparts, *Dt* in the a_1-*Dt* two-element system and *Ac* in the *Ds-Ac* two-element system, *Spm* does not show pronounced dosage effects that are reflected in altered frequencies or times of occurrence of mutation at the modified A_1 locus. Like other controlling elements, however, *Spm* undergoes transposition and consequently occupies no set position in the chromosome complement. An individual plant may have several *Spm* elements, each occupying a different site in the chromosome complement. Because it shows no dosage effects, the number of *Spm* elements present in the nuclei of a plant, as well as their locations within the chromosomes, must be determined by progeny tests.

In addition to the modifications affecting stable gene action, the element at A_1 also undergoes another type of change, but far less frequently. These changes, called "changes in state," are expressed by striking differences in the types of mutation that occur subsequently in the presence of *Spm*, and also in their times and frequencies of occurrence during development of each tissue. They also affect the degree of gene action that occurs in the absence of *Spm*. This varies among the states, and in this respect they form a graded series, from those that produce low levels of pigment intensity to those that give high levels. A few of the latter produce pigment intensities approximating that given by the genic materials at the standard A_1 locus. Nevertheless, with these latter states as with all states of a_1^{m-1} examined, pigment formation is completely suppressed in the presence of *Spm* and will appear only after the element located at A_1 undergoes some mutation-inducing event, or after *Spm* is removed from the nucleus. With respect to state it has also been found that the type of gene action that appears in the absence of *Spm* is not correlated with the types and patterns of mutation that appear in its presence. The states of a_1^{m-1} will be discussed more fully later.

Inheritance patterns of Spm. In Year Book No. 53, evidence was reported of linkage of *Spm* with *Y* (yellow endosperm), located in chromosome 6, in some plants of a particular culture (see table 17 on p. 257 of that Year Book). In these plants, only one *Spm* element was present. Their constitutions were a_1^{m-1} Sh_2/a_1 sh_2; *Y Spm*/*y* +. (The a_1 allele in these plants belongs to the a_1-*Dt* system of control of gene action. It does not respond to *Spm* and therefore behaves as a stable recessive in plants that have *Spm*. Shrunken endosperm, sh_2, is very closely linked to a_1 and shows less than one-quarter per cent crossing over with it.) When these plants were crossed by plants homozygous for a_1, sh_2, and *y* and having no *Spm*, the types of kernels on the resulting ears indicated that approximately 35 per cent crossing over had occurred between Y and *Spm* in the heterozygous parent plants. In the Sh_2 class there was a total of 1470 kernels. Of these, 723 were uniformly pigmented, showing a pale color in the aleurone layer (no *Spm* present); 269 of them were Y and 454 were *y*. In 740 of the Sh_2 kernels, spots of deep pigmentation appeared in a

colorless background (*Spm* present); 451 of these were Y and 289 were *y*. In addition, there were 7 completely colorless kernels; 4 were Y and 3 were *y*. Among the 1489 kernels in the sh_2 class, only 7 carried a_1^{m-1}; 3 of these were pale-colored (no *Spm*), and 4 had spots of deep color in a colorless background (*Spm* present). All other kernels in the sh_2 class had completely colorless aleurone; a 1:1 segregation for *Y* and *y* appeared among them. Plants were grown from selected kernels of all classes on these ears, and each was subjected to a particular set of tests. It was obvious that three major types of test were required: (1) verification of linkage of *Spm* with *Y* in plants derived from the variegated kernels in the Sh_2 *Y* class, (2) verification of the presence of a_1^{m-1} but the absence of *Spm* in plants derived from the uniformly pale-colored kernels, and (3) determination of whether or not *Spm* would be present in approximately 65 per cent of the plants derived from the a_1 sh_2 Y class of kernels and in approximately 35 per cent of the plants derived from the a_1 sh_2 *y* class of kernels.

Tests other than these three were also required. It was believed that somatic losses of *Spm* from some nuclei were occurring in some cases; this assumption was based on the presence in some plants carrying a_1^{m-1} and *Spm* of distinct sectors showing the phenotype that appears in the absence of *Spm*. Tests of the assumption could be readily carried out when such a sector extended into the tassel. Pollen collected from the sectorial and nonsectorial parts of the same tassel could be used in particularly designed test crosses (see below), which allowed detection of the presence or absence within the functional pollen grains of the *Spm* element and also of an a_1^{m-1} locus capable of responding to *Spm* in the expected manner. Such tests were made, and they fully confirmed the assumption that somatic losses of *Spm* from some nuclei occur during development.

In plants having one *Spm* element, transposition of *Spm* in some germinal cells can result not only in loss of *Spm* from some nuclei, as described above, but also in changes in its location, or increases in its number, in others. Since the rate of transposition of *Spm* appears to be relatively low, in view of the rather sharp linkage relations described, detection of cases of transposition required tests of relatively large numbers of individuals among the progeny of plants having one *Spm* element whose location was known. Such tests were conducted, and evidence of changes in location and increase in numbers of *Spm* elements was found.

Test (1) was extensive. It was accomplished by crossing each plant with one that either was homozygous for a_1^{m-1}, Sh_2, and *y* and had no *Spm* or was homozygous for a_1, sh_2, and *y* and had no *Spm*. For many plants both test crosses were made, and the results obtained in each test were the same except in a few instances where loss or change in position of *Spm* occurred early in an individual cell of the plant. Most of these were evident because of the fact that one of the ears produced by the plant was obviously sectorial with regard to *Spm* constitution. A plant homozygous of a_1^{m-1} but having no *Spm* is particularly useful for determining the presence or absence of *Spm* in another plant that is either a_1^{m-1}/a_1^{m-1}, a_1^{m-1}/a_1, or a_1/a_1 in constitution, and also for determining the numbers of *Spm* elements that may be present in such a plant. An intercross is made between the two plants. If *Spm* is present in the plant being tested, all the kernels that received it from gametes of this plant will show colored spots in a colorless background, and all those that did not receive it will be uniformly pale in color. With few exceptions, the ratio of variegated to pale kernels will indicate the numbers of *Spm* elements that were present in the zygote of this plant. The exceptions arise from early-occurring losses and transpositions of *Spm*, but the frequency of these is relatively low. If the tester

plant, which is homozygous for a_1^{m-1} and has no *Spm*, is also homozygous for some known recessive markers such as *wx*, *pr*, and *y*, and if the plant being tested is heterozygous for such markers, evidence of linkage of *Spm* to one or another of these markers, or evidence of absence of such linkage, is readily obtained.

Test (1), outlined above, verified the linkage of *Spm* with Y that had been observed in the parent plants, and the ratios of kernel types were the same as those shown by the parent plants. As expected, however, a few cases were encountered of change in location or increase in number of *Spm* elements, which had occurred in a germinal cell of the heterozygous parent plant. As an illustration of these tests, data obtained from fifty-six plants may be summarized. In forty-seven of them, linkage of *Spm* to Y was clearly expressed and to the same degree in each plant. Among the 7705 kernels in the pale class (no *Spm*), 2534 were Y and 5171 were *y*. Among the 7434 kernels in the variegated class (*Spm* present), 4862 were Y and 2572 were *y*. These data indicate that *Spm* is located approximately 35 crossover units from Y. In nine plants, the ratios of kernel types did not conform with this. In four of them, one *Spm* element was present but its linkage to Y was not expressed with certainty on any of the ears produced. Among a total of 1142 kernels on these ears, 533 had pale aleurone color (no *Spm*); 258 of them were Y and 275 were *y*. Among the 609 variegated kernels (*Spm*), 321 were Y and 288 were *y*. In two plants, two independently located *Spm* elements certainly were present. On each ear produced by these plants, a ratio of 1 kernel with no *Spm* to 3 kernels with *Spm* was observed. A total of 500 kernels was produced. In the class with pale-colored aleurone (no *Spm*), there were 117 kernels; 46 of them were Y and 71 were *y*. Among the 383 kernels in the variegated class (*Spm* present), 206 were Y and 177 were *y*. The data suggest that in both these plants one *Spm* element was carried in the Y chromosome and the other was located elsewhere. In the three remaining plants, the ratio of kernel types on the ears deviated in another way from that which might have been expected. Although the number of kernels on these ears was low, the deviation from a ratio of 1 *Spm* to 1 no-*Spm* was obvious: 27 to 70, 43 to 111, and 36 to 66. On none of these ears was there any evidence of linkage of *Spm* with *Y;* in the Y class there were 117 pale-colored kernels to 50 variegated kernels, and in the *y* class there were 129 pale-colored kernels to 58 variegated kernels. Frequent but late-occurring losses or transpositions of *Spm* may have been responsible for the observed deviation from the expected 1:1 ratio, although other causes may be considered. Progeny tests are required before any definite conclusions can be drawn regarding cause.

Test (3) is considered an important one because in the two classes involved, the presence or absence of *Spm* could not be determined by observation of type and distribution of anthocyanin pigmentation. All kernels were homozygous for a_1, and since this recessive allele of A_1 does not respond to *Spm*, anthocyanin pigment was absent in all kernels of these two classes. Tests for the presence or absence of *Spm* and for its location, if present, were conducted with fifty-six plants derived from the Y class of colorless, sh_2 kernels and with sixty plants derived from the *y* class of such kernels. Each plant was crossed by a plant homozygous for a_1^{m-1}, Sh_2, and *y* and having no *Spm*—the *Spm* tester stock described above. If no *Spm* was present in a plant being tested, all kernels on an ear resulting from this cross would be uniformly pale-colored. If one *Spm* element was present, half the kernels on an ear would be pale-colored (no *Spm*) and the other half would be variegated, with colored spots on a colorless background (*Spm* present). If more than one *Spm* was present, the ratio of variegated to pale kernels would be higher. With this mode of testing for *Spm*, it was possible to learn

that no *Spm* was present in twenty-four of the fifty-six plants derived from the Y class of kernels, and that in the remaining thirty-two plants one *Spm* element was present. Its linkage with Y was clearly expressed in thirty of these thirty-two plants. Among a total of 7792 kernels on the ears produced by the thirty plants, 3985 were uniformly pale-colored (no *Spm*); 1366 of them were Y and 2619 were *y*. The remaining 3807 kernels had a colorless background in which spots of deep color appeared (*Spm* present); 2472 of them were Y and 1335 were *y*. On the basis of these data *Spm* may be placed in chromosome 6, approximately 35 crossover units from Y. It will be noted that this is the same distance from Y indicated by the data from test (1), given above. The ratios of kernel types on ears produced by two of the thirty-two plants having one *Spm* element did not give clear evidence of linkage of *Spm* with Y. On one ear there were 70 pale-colored kernels and 88 variegated kernels. In the pale class, 30 were Y and 40 were *y;* in the variegated class, 47 were Y and 41 were *y*. On the ear of the other plant there were 123 pale kernels, 66 of which were Y and 57 *y*, and 154 variegated kernels, 79 of which were Y and 75 *y*.

Among the sixty tested plants derived from the a_1 sh_2 *y* class of kernels, seventeen had a single *Spm* element and forty-three had no *Spm*. On the ears produced by the seventeen plants having *Spm,* after the test cross described above, there was a total of 6746 kernels; 3465 of these were pale-colored (no *Spm*), and 3281 showed spots of deep color on a colorless background (*Spm* present).

These progeny tests again indicated that *Spm* was located in the Y-carrying chromosome 6 of the heterozygous parent plants, and again placed it approximately 35 crossover units from Y. In linkage studies with transposable elements, an error is always introduced into the calculations of crossover distances, and the degree of this is related to the frequency of occurrence of transposition of the element, before gamete formation, to new locations in the chromosome complement. As may be noted from the several tests outlined above, this frequency in the case of *Spm* is not great enough to have a serious effect on determinations of linkage relationships.

Test (2), mentioned earlier, was readily conducted, in several different ways. The plants being tested were assumed to be a_1^{m-1} Sh_2/a_1 sh_2 in constitution, and to have no *Spm*. The absence of *Spm* was confirmed in all cases by means of the test cross outlined above. The presence of a_1^{m-1}, carried in the Sh_2 chromosome and capable of responding to *Spm,* was readily determined by crossing these plants to plants homozygous for a_1 and sh_2, some carrying an *Spm* element and others lacking this element. On the ears produced by the latter cross, nearly all the Sh_2 kernels were pale-colored and, as expected, nearly all the sh_2 kernels were colorless; no variegated kernels appeared. On the ears produced by the former cross, however, the two expected classes of kernels appeared in the Sh_2 class: those showing spots of deep color on a colorless background (in which both a_1^{m-1} and *Spm* were present), and those showing a uniformly pale color (in which a_1^{m-1} was present but *Spm* was absent). Also, as expected, nearly all the sh_2 kernels were colorless. The location of *Spm* in the a_1 sh_2 parent was known in some cases, and the expected linkage with factors carried in the chromosome that also had *Spm* was made evident on the ears that resulted from their use in these crosses.

The tests outlined above have been described here in some detail in order to indicate the necessary initial analytical methods in an investigation of the basic mode of operation of this two-element system. With the general mode of operation defined, it was possible to conduct a number of further tests. Some of these were designed to determine the number of *Spm* elements present in individual plants of a particular progeny, when the presence of

two or more was suspected in the parent plant. Others were aimed at determining various locations in the chromosome complement that may be occupied by *Spm*. At present, two positions in chromosome 6, two in chromosome 5, and two in chromosome 9 have been identified. *Spm* also occupies other sites in the chromosome complement that have not yet been located. In another series of tests, individuals having two *Spm* elements, located at allelic positions in a pair of homologues, were tested in order to determine the frequency of loss of *Spm* from the female germ cells. It was found to be absent in approximately 6 to 10 per cent of the female gametes produced by these plants. The majority of such losses of *Spm* occurred late in the development of the germinal tissue.

In addition to the tests just discussed, an extensive series of tests was also conducted with each of eight distinctly different states of a_1^{m-1}. This was done in order to examine the mode of control of change in gene action at A_1 exhibited by each state in the presence of *Spm*, to discover the type of gene action appearing in its absence, and to determine the stability of each state—that is, its constancy—in the presence of *Spm*. Also, several of the states were combined in a single individual, and the independence of action of each in the presence of *Spm* was determined. Allelic relationships of states were revealed by segregation ratios in the progeny of these individuals.

The states of a_1^{m-1} and their significance. From an examination of the various states of a_1^{m-1} it has been possible to learn about the modes of control exerted by this a_1^{m-1}-*Spm* system on mutation types, frequencies of occurrence, and times of occurrence during the development of a tissue. Control of all these resides in the element located at A_1, and this is not influenced by the number of *Spm* elements that may be present, although *Spm* is required for the manifestation of these controlled types of expression. Seven of the eight different states that have been studied were isolated after a change that occurred in the element originally introduced at the A_1 locus. This original state of a_1^{m-1} gives rise, in the presence of *Spm*, to many early-occurring mutations, both in the plant tissues and in the aleurone layer of the kernel. The intensity of pigmentation these mutations produce ranges from faint to deep. A number of germinal mutations also occur, and these give rise to alleles that are stable in the presence of *Spm*. When plants having this state of a_1^{m-1} are crossed with plants homozygous for a_1, kernels on the resulting ears will occasionally show a decidedly modified pattern of mutation. The kinds of mutation may be altered, or their frequency of occurrence may be different, or their times of occurrence may be shifted; or combinations of these several identifiable alterations of expression may appear in such kernels. Some of these kernels were removed from ears, and plants were grown from them. These plants, in turn, were examined to determine the behavior of a_1^{m-1} in them. It was found that in the presence of *Spm* the pattern of mutation appearing in the kernel from which the plant arose reappeared in the following generation. In other words, the alteration at a_1^{m-1} responsible for the changed pattern of mutation was maintained in chromosome reduplication and thus was heritable.

A description of several of the derived states may be used to illustrate the range of their expressions. One state produces, in the presence of *Spm*, only a relatively few dots of color in an otherwise colorless kernel, but these dots are intensely pigmented. The plants also show only a few small streaks of deep pigmentation in a nonpigmented background. In the absence of *Spm*, the plants are darkly pigmented but the kernels are only faintly colored, and these expressions are constant in successive generations as long as *Spm* is absent. When *Spm* is returned to the nucleus, the pattern of expression described above again appears—a few dots of deep pigmentation in a colorless background in the kernel and

a few streaks of deep pigmentation in the plant. Only very rarely does this state of a_1^{m-1} give rise to a mutation in a germinal cell, and no subsequent change of this state to another state has yet been identified.

Another state, derived from the original one, is somewhat similar to that just described in its behavior in the presence of *Spm*. In the kernels, dots of deep color appear in a colorless background, but their number is larger. On an occasional kernel, a large deeply pigmented spot may also be present. The plants having this state show a number of fine streaks of deep pigmentation in a nonpigmented background. In the absence of *Spm*, however, this state is readily distinguished from the one just described, for now both the kernels and the plants are intensely pigmented. Return of *Spm* to the nucleus brings back its suppressor action and calls forth the pattern of mutation characteristic of this state. Few germinal mutations occur, and changes of this state to another state are rare.

Still another state gives rise to dots of deep color in the presence of *Spm*, but these are so numerous that the kernel appears almost solidly colored when viewed from a distance. The plant also shows numerous small streaks containing anthocyanin pigment. Only a few germinal mutations occur. In the absence of *Spm*, the kernels are lightly but distinctly pigmented, and the plants are also pigmented. Another state gives rise, in the presence of *Spm*, to many early-occurring mutations, and these express the higher levels of pigment intensity. Many germinal mutations occur. In the absence of *Spm*, the kernels having this state are lightly pigmented and the plants also are pigmented. In this case, as with all states so far examined, return of *Spm* by appropriate crosses in some succeeding generation will call forth the pattern of mutation characteristic of the state.

One state differs from all others with respect to the types of mutation it produces. In the presence of *Spm*, the mutations may occur early in development. In the kernels, the colored patches, representing mutant areas, show low levels of pigment intensity. Only very rarely, indeed, does a colored patch appear that expresses the standard A_1 phenotype. Many germinal mutations occur, and among the kernels having them the same low levels of pigment intensity are shown. The plants derived from these kernels are pigmented, the intensity in each case corresponding to that shown by the kernel from which the plant arose. In the absence of *Spm*, the kernels having this state show either no color at all or only a very faint trace at their base. In the plants, also, no anthocyanin pigment is detected on visual examination.

By intercrosses, it is possible to combine two different states in an individual plant or kernel. When *Spm* is present, the mutation pattern produced by each of the states is evident, indicating the autonomy of each with respect to its mode of action. This is well illustrated in kernels having the state just described and also a state that gives only late-occurring mutations that are expressed in the kernel as deep-colored dots. Both patterns of mutation appear in these kernels: the pale-colored areas, many of which are large, produced by the former state, and the deep-colored dots produced by the latter. There appears to be no interaction between the states that affects their individual modes of expression. The autonomy of each state is also made evident by the ratio of types of kernels that appear on ears produced when plants having two different states of a_1^{m-1} are crossed to plants homozygous for a_1. The two states segregate from each other at meiosis, and a 1:1 ratio appears among the kernels.

Conclusions regarding the operation of the a_1^{m-1}-Spm system. The general mode of control of gene action and of mutation by this a_1^{m-1}-*Spm* two-element system is now evident. The element of this system that is inserted at the A_1 locus plays a major part in controlling gene action and

in effecting changes in such action, both in the presence and in the absence of the second element, *Spm*. The *Spm* element exerts a direct influence on the element located at A_1, in two distinctly different ways. First, in the absence of *Spm* some gene action occurs at A_1, but in its presence this is totally suppressed. Secondly, *Spm* induces modifications of the element residing at A_1 that do not occur in its absence. Two kinds of modification arise. One effects a stable mutation, and the mutants so formed give rise to a series of alleles that differ from one another both quantitatively and qualitatively. The second type of modification, of rarer occurrence, leads to a change in the controlling element at A_1—a change in state—that is subsequently discerned. In the presence of *Spm*, these modifications are expressed by changes in the kinds of mutations that occur, their frequencies of occurrence, and their times of occurrence during the development of the tissues. These modifications of state also affect the degree of action of the genic materials at the A_1 locus in the absence of *Spm*.

Spm may be transposed from one location to another in the chromosome complement, both in somatic and in germinal cells, without losing its specificity of action in the process. Thus, loss of *Spm* from some nuclei and increase in others may occur within an individual plant. An increased number of *Spm* elements in a nucleus is not made evident by changed patterns of mutation at a_1^{m-1}. This contrasts greatly with the case of the a_1-*Dt* system, where increase in number of *Dt* elements is made evident by increased frequencies of occurrence of mutation at the a_1 locus. It also contrasts with the behavior of *Ac* in the *Ds-Ac* system. With regard to a_1^{m-3} and a_1^{m-4}, both of which express control of mutation at A_1 by the *Ds-Ac* system, successive increases in number of *Ac* elements retard in a stepwise manner the time of occurrence of mutation at the modified A_1 locus in each case.

Knowledge gained from an examination of the mode of operation of this system of control of gene action and mutation, and a comparison with other systems operating at the very same locus, has greatly enlarged our appreciation of the probable significance of such systems in regulating gene action during development. Somatically occurring changes in gene action, both gross and subtle, can result from their operation, and these are well regulated with regard to both time of occurrence and type of change.

Continued Studies of the Mode of Operation of the Controlling Elements *Ds* and *Ac*

Several other projects were carried out during the year. Two of them were concerned with the elements *Ds* and *Ac*. The general mode of behavior of these two elements has been described in many previous reports. Although the tests conducted this year were many and the data obtained were extensive, only the most significant evidence and conclusions will be given here.

The first of these projects was an analysis of the direct control by *Ac* of change in gene action at the bronze (*bz*) locus in chromosome 9 when it is inserted there. It could be demonstrated that these changes are associated with events affecting the *Ac* element itself. They give rise to several different phenotypic expressions of the genic materials at the bronze locus: a stable recessive (*bz*) expression, a full dominant (*Bz*) expression, and an expression that is intermediate between these two extremes. Stability of the mutants depends on whether or not *Ac* is removed from the immediate vicinity of the bronze locus by the event that affects it and results in the change in genic expression. If it is removed, the mutant is stable in subsequent generations. If it remains, the mutant is unstable, in that subsequent alterations of *Ac* may lead to further change in action of the genic materials at the locus. The time of occurrence, during the devel-

opment of a tissue, of these mutation-inducing alterations of *Ac* depends on the total dose of *Ac* present in the nucleus: the higher the dose, the later they will occur. Such responses to *Ac* dose may be effected either by increasing the number of chromosomes 9 carrying *Ac* at the bronze locus—from 1 to 3 in the kernel and from 1 to 2 in the plant—or by adding *Ac* elements that are located elsewhere in the complement when only one chromosome 9 carries *Ac* at the bronze locus.

Some of these *Ac*-altering events at the bronze locus give rise to a dicentric chromatid and the corresponding acentric fragment. Changes in the frequency of occurrence of this type of event characterize some of the changes in state of *Ac*. One other modification was detected, and it is of general significance in considering interrelations that may arise between controlling elements. One kernel was found that showed a marked increase in frequency of occurrence of mutation to *Bz*. The tissues of the plant grown from it exhibited the same increased frequency. Tests of this plant revealed that an alteration had occurred at the bronze locus in a germinal cell of the parent plant, resulting in a modification of the mode of control of subsequent mutation. *Ac* was still present and was required for the occurrence of these mutations, but it was no longer located at the bronze locus in the short arm of chromosome 9. The mode of control now followed that which characterizes the *Ds-Ac* two-element system, in which the *Ds* element directly controls the change in gene action at the locus where it resides, and the *Ac* element governs the occurrence of these mutation-inducing events at *Ds*. It must be concluded, therefore, that this modification arose from substitution of a *Ds* element for the *Ac* element at the bronze locus; or that the *Ac* element originally inserted there is compound and may be separated into two components, a *Ds* and an *Ac* element; or, possibly, that a *Ds* element may originate from some modification of an *Ac* element. No evidence is now available to suggest which of these alternatives is most probable. Nevertheless, the observed change from a one-element to a two-element system of control of gene action and mutation is significant in considering the relations that exist between controlling elements and systems of such elements.

An additional project that received much study was concerned with the behavior of *Ds* after its insertion just to the left of Sh_1 in chromosome 9. In this position, it induces changes in action of genic substances located on either side of it, and these effects may include a segment of the chromosome six or more crossover units long in the standard chromosome 9. During the past year, several cases of extended modification of gene action, in which the genic components farthest removed from *Ds* exhibited reversion to standard expression, were examined. In all cases, it could be determined that the *Ds* element was a component of the segment of chromosome showing altered genic action, and that the reversions observed were accomplished by some change involving the *Ds* element itself. The patterns of reversion were those associated with the operation of the two elements, *Ds* and *Ac*: *Ac* was required for their occurrence, and the times of occurrence reflected the dose of *Ac* that was present in the nucleus. In the cases examined, the reversions to normal action of a genic component within the modified segment were not accompanied by loss of *Ds* or by change in its location. This is unlike the behavior of *Ds* at some other known loci. In these other cases, it has been determined that reversion of gene action is often accompanied by removal of *Ds* from the immediate vicinity of the locus concerned.

Many different tests have been made of the behavior of *Ds* when inserted just to the left of Sh_1, and all of them indicate that *Ds* is effectively fixed in location after its insertion there. It can continue, then, to exert its influence on the action of genic substances located to either side of it. Thus a sequential series of changes in action of

these genic materials can occur as long as *Ac* is also present in the nucleus, and a number of such sequences have been followed through three or more steps. The kinds of modification in gene action induced by *Ds* at this location resemble, in some respects, those appearing spontaneously at some other well-known gene loci in maize, such as the *R* locus, whose numerous alleles are known, as well as spontaneous rates of change to other alleles. It is possible that there is a controlling element or elements present at this locus, and also at other loci in the standard chromosome complement of maize, and that the modifications in gene action they induce are responsible for the appearance of the mutants and for the particular sequences of change from one type of allele to another that have been observed.